SEQUENCE MATCHING, SIMPLE SEARCHING

PGA Course in Bioinformatics
Tools for Comparative Analysis
April 29, 2002

Outline

- Sequence alignment algorithms
  - Rigorous Optimality:Needleman-Wunsch and Smith-Waterman
  - Rapid, heuristic algorithms
    - BLAST
    - FASTA
    - and their relatives
- Databases and Search Tools

MAJOR SITES WE WILL USE

- http://www.ncbi.nlm.nih.gov/
- http://workbench.sdsc.edu

MEDICAL SUBJECT HEADINGS

- CONTROLLED Vocabulary
- Indexing of articles, books, etc.
- Current version has over 300,000 terms
- Can download list and make your own assortment

Needleman Wunsch Algorithm

- Global alignment
- Guaranteed to calculate an Optimal similarity score
- Begin at the beginning of each sequence and go to the end.
- Cannot detect domains

Smith-Waterman Algorithm

- Optimal Local Alignment
- Guaranteed to find all significant matches to a given query
- Takes the query sequence versus every sequence in the database
- Can be used with arbitrary scoring systems
- **COMPUTATIONALLY EXPENSIVE!!!**

Scoring Matrices

- Relatively simple for DNA-gap penalties or mismatches-can be made to look at Pu/Py
- Protein matches look also at similarity (leu/ileu)

Protein Scoring Matrices

- Chemical similarity: 210 pairs of aa
- Nearness in Genetic Code
- Chemical similarity, e.g., hydrophobicity
- Observed Substitution Schemes

Observed AA Substitution Matrices

- PAM
- BLOSUM

PAM: Point Accepted Mutation

- DAYHOFF et al.
- Residue replacement in related proteins
- A model of molecular evolution
- 1 PAM = average change in 1% of all amino acid possibilities
- 100 PAMs does not mean every residue is changed.

PAM continued

- TIME is NOT correlated with PAM

Means different families of proteins evolve at different rates

BLOSUM

- Block Substitution Matrix
- Henikoff and Henikoff, PNAS, 1992
- Number following indicates per cent identity within set, BLOSUM62=62% id
- Finds short, highly similar sequences

BLAST-Basic Local Alignment Sequence Tool

- Objective: find all local regions of similarity distinguishable from random
- Only local alignments permitted,
- Gaps permitted in version 2
- Statistically sound (Karlin and Altschul), but no guarantee of optimality

BLAST: Three Step Algorithm

- Compile a list of high scoring words of length w (w=4 for proteins, 12 for nucleic acids)
- Scan for word hits of score greater than threshold, T
- Extend word hit in both directions to find High Scoring Pairs with scores greater than S

Other BLAST Programs

- BLASTN: nucleic acid query to NA database
- BLSATP: Protein query to Protein database
- BLASTX: Translated nucleic acid query to Protein database
- TBLASTN: Protein query against (translated) nucleic acid database
- TBLASTX: Translated nucleic acid against translated nucleic acid database

OTHER BLAST VARIATIONS

- Gapped BLAST (BLAST 2.0) -extend words from no-gap to gap, generate gapped alignments
- PSI-BLAST- Position Specific Iterated BLAST-use gapped BLAST, generate a Profile from multiple iterations used instead of the input and Distance Matrix

Limitations to BLAST

- Needs islands of strong homology
- Limits on the combination of scoring and penalty values
- The variants (blastx, tblastn, tblastx) use 6-frame translation-miss sequences with frameshifts)
- Finds and reports ONLY local alignments

A WALK THROUGH BLAST

BLAST RULES OF THUMB

- For short amino acid sequences (20-40), 50% identity happens by chance
- If A and B are homologous, and B and C are homologous, then A and C are, even if you can't see it.
- You can get similarity in the absence of homology for low complexity, transmembrane and coiled-coil regions. These have to be eliminated by you.

BLAST Significance

- If you change scoring systems, you can still compare search results if you normalize the score.

  S'=(lambdaS-lnK)/ln2. Lambda and K are associated with the scoring system.

  S', with a given E, is significant if it is greater than N/E, N the size of the search space.

FASTA: FAST Alignment

- http://alpha10.bioch.virginia.edu/fasta/
- http://www2.ebi.ac.uk/fasta3
- http://workbench.sdsc.edu

- Rapid Global alignment

- Not a strong mathematical basis

FASTA: WHY USE IT?

- Allow alignments to shift frames

LALIGN

- Essentially a FASTA derivative for local alignments
- Compares two proteins to identify regions of similarity
- Will report several sequence alignments within a given sequence
- Works for internal repeats that are missed by FASTA because of gaps.

SITEs for LALIGN

- http://fasta.bioch.virginia.edu/fasta/lalign.htm
- http://xylian.igh.cnrs.fr/bin/lalign-guess.cgi
- http://biowb.sdsc.edu (registration necessary but painless)
- PALIGN http://fasta.bioch.virginia.edu/fasta/palign.htm (plots a graph of the areas of alignment)

ENTREZ: Linked Databases

http://www.ncbi.nlm.nih.gov/Entrez/

- Concept of Neighbor-usually BLAST relationship
- Precomputed=Fast
- Related sequence, structure neighbors, related articles

EST DATABASES:Quality issues

- SEQUENCE QUALITY
  - calculated error less than 1% (Phred-20) is the rule
  - frameshifts and stops common
  - Rules are usually observed by exception
  - There are lots of exceptions in the public data
  - Many 3' UTRs

EST Databases: Quality #2

- CLONE QUALITY
  - Over-representation
  - Tissue specificity
  - Developmental stage specificity
  - Unprocessed mRNA clones
  - Chimeras
  - Contamination

EST Cluster Databases

- STACK-at SANBI http://sanbi.ac.za
- TIGR-animals, plants, other http://www.tigr.org/tdb/tgi.shtml
- Unigene-NCBI
  - Human, mouse, rat, cow,zebrafish
  - mRNAs
  - predicted mRNAs

UNIGENE

- A LIST OF LISTS
  - The cluster and known EST, mRNA pieces
  - Additional annotation-gene name, etc.
  - Distributed as a subset of dbest

NOT included in the BLAST searchable DB at NCBI

Caveats on Clusters

- Not stable
- Can go to complete cDNAs as available

LOCUSLINK

(http://www.ncbi.nlm.nih.gov/LocusLink)

- A useful, searchable compendium of loci across human, mouse, rat, Drosophila and zebrafish
- Linked for PubMed, OMIM, RefSeq, Homologene data, Unigene, and Variation Data

Resources for Genomic Comparison

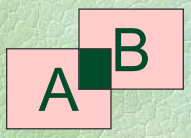
- GLASS-http://plover.lcs.mit.edu
- PipMaker: http://bio.cse.psu.edu
- Rosetta: http:// plover.lcs.mit.edu(genes)
- SGP: htttp://soft.ice.mpg.de/sgp-1
- VISTA: http://www-gsd.lbl.gov/VISTA
- WABA: http://www.cse.ucsc.edu/~kent/xenoAli/index.html

EFFICIENT SEARCHING

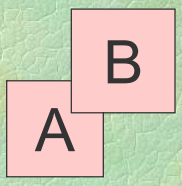
- Use Wild Cards: #,$,?,*
- Use Boolean Operators
  - Not
  - And
  - Or
  - Nor

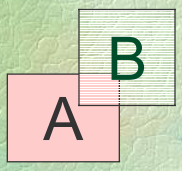
Boolean Operators

- *AND* A and B BOTH
- *OR* A or B EITHER
- *NOT* B not A Have B, do not have A
- *NOR* A nor B A but not B OR B but not A



and





A not B

or

WILD CARDS

- Match one character-NCBI uses #
- Match zero or one character NCBI uses $, others ?
- Match zero or more characters-usually *

RULES OF THUMB

- Use an up-to-date database; repeat often
- Choose a fast algorithm
- Use the most recent version
- Work at the protein level--for a small amount of evolutionary change, DNA sequence contains less information about homology
- Respect your own ***intuition***

Other Resources

- NCBI Education Page http://www.ncbi.nlm.nih.gov/Education/index.html
- BCM Gene Finder http://searchlauncher.bcm.tmc.edu/docs/sl_links.html
- EBI-SwissProt, TrEMBL, PIR, SRS, Tools http://www.ebi.ac.uk
- ExPASy-SwissProt, TrEMBL http://www.expasy.ch/
- DISC-DNA Information and Stock Center http://www.dna.affrc.go.jp